UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUN 18 2002

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Science Review in Support of a Label Amendment and a Petition (Petition No. 0F06144) for an Exemption From the Requirements of Tolerances for EthylBloc™ (EPA Reg. No. 071297-1) Containing 0.14% 1-Methylcyclopropene (Chemical No. 224459). Review of Acute and Subchronic Toxicity Studies and Other Human Health Data/Information. DP Barcode D281146; Case No. 063215; Submission No. S610458; MRIDs 456090-01 to -04.

FROM: Russell S. Jones, Ph.D., Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

TO: Driss Benmhend, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

ACTION REQUESTED

AgroFresh, Inc. [formerly BioTechnologies for Horticulture, Inc (BTH, Inc.; a subsidiary of Rohm and Haas Company)] has submitted an apple residue study, a new subchronic inhalation toxicity study, an analytical method for the determination of a manufacturing impurity, and an updated risk assessment in support of: (i) a petition (Petition No. 0F06144) for an exemption from the requirements of tolerances for residues of 1-MCP on stored food commodities; and (ii) a label amendment to add indoor use on post-harvested fruits and vegetables (MRIDs 456090-01 to -04).

1-MCP is the active ingredient in the end-use product, EthylBloc™ which contains 0.14% 1-MCP. EthylBloc™ is a powdered product that releases 1-MCP as a gas when mixed with water or a buffering agent. The end-use product is currently registered for non-food use on floral and nursery crops.

CONCLUSIONS AND RECOMMENDATIONS

1. The submitted analytical methods/chemistry, residue chemistry, and subchronic inhalation toxicity studies are acceptable and support the label amendment request for

EthylBloc™ (EPA Reg. No. 071297-1) and the tolerance exemption petition for 1-MCP (Petition No. 0F06144).

2. BPPD concurs with the dietary and worker risk assessment submitted by the registrant (see below) for 1-MCP and CMP. When the product is used according to label directions, there is virtually no risk to consumers of treated food matrices or to workers handling the product formulations [see also the review of Dietary and Worker Risk Assessment (MRID 453803-07) in the Memorandum from R. S. Jones to D. Benmhend, dated 1/30/2002). It is highly unlikely that workers and consumers will be exposed to 1-MCP and/or CMP when the product is used according to proposed label use directions.

3. The petition for an exemption from the requirements of tolerances for 1-MCP on food, and the request for a label amendment to permit use of the product on food, are supported by the studies/data reviewed in this document and by previously submitted studies/data (see Memoranda from R. S. Jones to D. Benmhend, dated 12/23/1998; 3/9/2000; 9/28/2000; 2/21/2001; 3/21/2001; 5/3/2001; and 1/30/2002).

STUDY SUMMARIES

Product Chemistry (Non-Guideline Study)

Determination of 3-Chloro-2-Methylpropene Concentration in Chamber Treated with 1-Methylcyclopropene (MRID 456090-03): A study was designed to: (i) evaluate an analytical method for the analysis and quantitation of 3-chloro-2-methylpropene (CMP; a potential impurity in EthylBloc®) in air samples; and (ii) determine the concentration of CMP in a chamber treated with EthylBloc®, [containing 0.14%1-methylcyclopropene (1-MCP) as its active ingredient] under simulated commercial conditions. Concentrations of CMP were analyzed with a new GC/MS analytical method with an LOQ of 0.000407 ppm CMP (for a 48 L air sample). The GC/MS method for determining CMP was shown to be accurate and precise. A potential interfering substance (a CMP isomer) was shown to be adequately resolved and separated from CMP by the GC/MS method. Concentrations of 1-MCP were determined by GC/FID using a previously reviewed method (with minor revisions). Representative chromatograms, mass spectra, examples of calculations, and other raw data were presented by the registrant and adequately supported the analytical methods for determining 1-MCP and CMP in air samples under simulated commercial conditions.

In the simulation study, a prototype of a commercial generator was used to release 1-MCP into a test chamber (a sealed truck trailer) to achieve an air concentration of approximately 1 ppm 1-MCP. The study was conducted three times on three consecutive days. Samples of 1-MCP were collected hourly. Due to the extremely low levels of CMP, air samples for CMP analysis were collected by drawing gas through a trapping

medium continuously from the trailer for 1 to 3 hours; the trapping medium was subsequently desorbed with carbon disulfide prior to analysis. The mean concentration of 1-MCP (n = 26) for all three sampling intervals was 0.82 ± 0.1 ppm. The mean concentration of CMP (n = 9) was 0.00052 ± 0.00028 ppm for all three sampling intervals; six of the CMP observations were below the LOQ and were assigned a value of one-half the LOQ. There were no statistical differences in respective1-MCP or CMP concentrations between studies. The ratio of mean CMP concentration to mean 1-MCP concentration was approximately 1:1577 or 0.063% (0.00052 ppm CMP/0.82 ppm 1-MCP). Neither 1-MCP nor CMP were detected in control samples collected from the treatment chamber prior to the initiation of the experiments.

Classification: Acceptable.

Magnitude of the Residue (OPPTS 860.1000 and 860.1500)

^{14}C-1-Methylcyclopropene (1-MCP) :Apple Residue Study (MRID 456090-02): Total radioactive residues (TRR) were determined in four varieties of commercially-important apples (Red Delicious, Fuji, Gala, and Granny Smith) following fumigation treatment of with 1.2 ppm (v/v) of ^{14}C-1-MCP (1.2x the maximum proposed label rate) under simulated commercial treatment and storage conditions. Each apple was treated twice each at two different temperatures (0-3°C and ambient) in an airtight chamber for 24 hours, after which the chamber was vented. Apple samples were removed periodically (0 and 4 hours posttreatment, and up to 14 days posttreatment, depending on variety and temperature) for TRR analysis. A sample set consisted of three apples sampled from the top, middle, and bottom of the apple stack in the chamber, an untreated control and two fortified controls. In a second experiment, Red Delicious apples were exposed to 1.2 ppm ^{14}C-1-MCP for one week to observe the effects of extended treatment time (7X greater than label use directions) on apple residue levels. The TRR was determined by liquid scintillation counting (LSC). The measured limit of quantitation (LOQ) for the analytical method was 0.001 mg/kg and calculated limit of detection (LOD) was 0.0003 mg/kg. The average fortification recovery for sample sets in the main study was 82.9 ± 8.9% (range: 75.6-103.2%) with a relative standard deviation of 10.7% (n=39). The average fortification recovery for sample sets in the extended exposure study was 80.7 ± 2.9% (range: 78.3-83.9%) with a relative standard deviation of 36% (n=4). Residue levels were calculated as mg/kg equivalents of 1-MCP; analytical data were not corrected for method recoveries.

In the main residue study, average TRR for the eight treatments (four varieties at two temperatures; n=60) was 0.00391 mg/kg (range: 0.00114-0.00911 mg/kg). There were no trends of increasing or decreasing TRR with sampling interval in any apple variety. The TRR in apples treated and stored at ambient temperatures were generally higher than apples treated and stored at 0° C. The highest mean TRR was observed in Gala apples

(0.00540 ± 0.00293 mg/kg) treated and stored at ambient temperature. The lowest mean TRR was observed in Red Delicious apples (0.00310 ± 0.00108 mg/kg) treated and stored at 0-3°C.

In the extended exposure study, Red Delicious apples exposed to 1.2 ppm for 7 days (sampled and analyzed as described above), had mean TRR of 0.00693 mg/kg (range: 0.00656-0.00775 mg/kg) at 0 and 48 hours posttreatment. Mean TRR in the extended exposure study were 1.8x the mean TRR of the main residue study.

Classification: Acceptable.

90-Day Inhalation Toxicity (OPPTS 870.3465)

1-Methylcyclopropene: Three-Month Inhalation (Whole Body) Toxicity Study in Rats (MRID 456090-01): In a 90-day inhalation toxicity study, groups of young adult rats (10/sex/group) were exposed to atmospheric concentrations of 1-methylcyclopropene (1-MCP) by whole-body inhalation for 6 hours/day, 5 days/weeks over the course of 13 consecutive weeks (total of 67 exposures). Measured exposure concentrations of 1-MCP were 0, 24 ppm (S.D. ± 5.0 ppm), 107 ppm (S.D. ± 7.4 ppm), or 1031 ppm (S.D. ± 41.1 ppm) in whole-body chambers under dynamic conditions (target 1-MCP concentrations were 0, 20, 100, and 1000 ppm). After 13 weeks of exposure, rats were sacrificed and necropsied. There were no treatment-related mortalities, or other clinical signs of toxicity (other than increased salivation in rats treated at 1031 ppm), throughout the study. Histopathology demonstrated changes in the kidney (increased "intracytoplasmic eosinophilic structures consistent with hyaline droplets in the epithelium of the cortical tubules) and spleen (increased hemosiderin and red pulp congestion; and indications of α-2-microglobulin-type degeneration). Mild degenerative anemia was observed at 1031 ppm. Based on the data:

the 90-day inhalation NOAEL = 24 ppm and the LOAEL = 107 ppm.

Classification: Acceptable. A detailed discussion of 1-MCP chronic inhalation effects on rats is presented in the attached DER for MRID 456090-02.

Inhalation Developmental Study (OPPTS 870.3600)

1-Methylcyclopropene: Inhalation (Whole Body) Developmental Toxicity Study in Rats (MRID 456090-08): Groups of young adult pregnant rats were exposed to atmospheric concentrations of 107-1029 ppm 1-methylcyclopropene (1-MCP) by whole-body inhalation on days 6 to 19 of gestation. All rats survived the study and there were no signs of clinical toxicity at any dose level. Slight maternal toxicity (reduced body weight

gain; increased incidence of enlarged spleens) were observed at the mid- and high doses. No compound-related effects were observed on corpra lutea, implantations, litter sizes, live fetuses, early/late resorptions, fetal body weights, sex ratio, and the percentage of resorbed conceptuses. There were no observable effects on type/incidence of gross external soft tissue or skeletal effects ant any dose. Based on the data:

the inhalation developmental NOEL is >1029 ppm (543 mg/kg/day); and
the rat maternal toxicity NOEL = 107 ppm (56 mg/kg/day).

Classification: Acceptable. A detailed discussion of 1-MCP inhalation developmental and toxicity effects on pregnant rats is presented in the attached DER for MRID 456090-08.

NOTE: Above is a summary only. DER and Cover memo (by R. Gardner) are subject of another review.

Worker and Dietary Risk Assessment (Non-Guideline Study)

I. 1-Methylcyclopropene (1-MCP): Assessment of Worker and Consumer Risk (MRID 453803-07): The report begins with an Introduction containing a description of the uses of 1-MCP-containing products [EthylBloc® Technology (EPA Reg. No. 071297-1) and Smart Fresh™], and 1-MCP mode of action. This information has been discussed in detail in previous Memoranda for this active ingredient. The Introduction is followed by a review of 1-MCP toxicity studies. The data from these toxicity studies are listed in Table 1 below, together with appropriate data from previously reviewed toxicity studies (see Memorandum from R. S. Jones to D. Benmhend, dated 12/23/1998).

Table 1. The toxicity profile of 1-MCP (NOTE: except for the 90-day rat inhalation study and the inhalation developmental study, data were obtained from DERs attached to Memoranda from R. S. Jones to D. Benmhend, dated 12/23/1998 and 1/30/2002)

Study Type (OPPTS No.)	Dose/Concentration/Effect	Tox Category	MRID(s)
Acute Oral Toxicity (OPPTS 870.1100)	Rat LD_{50} >5000 mg/kg; Tox IV	IV	453803-08[1] 444647-05[2]
Acute Dermal Toxicity (OPPTS 870.1200)	Rabbit LC_{50} > 2000 mg/kg	III	444647-05[2]
Acute Inhalation Toxicity (OPPTS 870.1300)	Rat LC_{50} >1126 ppm (> 2.5 mg a.i./L)	IV	453803-01[1]
Primary Eye Irritation (OPPTS 870.2400)	Slight (Rabbit)	III	444647-07[2]
Primary Dermal Irritation (OPPTS 870.2500)	Not an irritant (Rabbit)	IV	444647-08[2]

Study Type (OPPTS No.)	Dose/Concentration/Effect	Tox Category	MRID(s)
Dermal Sensitization (OPPTS 870.2600)	Not a sensitizer (Guinea Pig)	IV	445170-05[2]
2-Week Rat Inhalation (Non-Guideline)	NOAEL = 100 ppm; (0.221 mg/L = 63 mg/kg-day) LOAEL = 300 ppm	-	453803-06[1]
90-Day Rat Inhalation (OPPTS 870.3465)	NOAEL = 24 ppm; (9-15 mg/kg/day) LOAEL = 107 ppm	-	456090-01
Inhalation Developmental (rat) (OPPTS 870.3600)	NOEL >1029 ppm (543 mg/kg/day) NOEL (maternal toxicity) = 107 ppm	-	456090-08
Bacterial Reverse Mutation Assay (Ames Assay; OPPTS 870.5100)	Negative at up to 1000 ppm 1-MCP in atmosphere	-	453803-02[1]
In vitro Mammalian Chromosome Aberration with Human Lymphocytes (CHO/HGPRT; OPPTS 870.5375)	Negative at up to 1000 ppm 1-MCP in atmosphere	-	453803-04[1]
In vivo Mammalian (Mouse) Micronucleus Test (OPPTS 870.5395)	Negative at up to 1000 ppm 1-MCP in atmosphere	-	453803-05[1]

[1] See Memorandum from R. S. Jones to D. Benmhend, dated 1/30/2002.
[2] See Memorandum from R. S. Jones to D. Benmhend, dated 12/23/1998.

EPA Reviewer's Note: The study author incorrectly states that the acute dermal LD_{50} for 1-MCP is >5000 mg/kg see MRID 456090-04, p. 7). According to the DER for MRID 444647-05, the correct value is LD_{50} >2000 mg/kg (see Memorandum from R. S. Jones to D. Benmhend, dated 12/23/1998); therefore, Toxicity category III is appropriate for acute dermal toxicity. No dermal toxicity studies using a limit dose of 5000 mg1-MCP/kg were available for review.

II. Worker Risk to 1-MCP and CMP: Worker risk was assessed by assuming two "worst-case" scenarios for inhalation to exposure 1-MCP: a "short-term scenario" and a "long-term" scenario. A "short-term" scenario was also used to assess worker risk for inhalation exposure to CMP. These scenarios, the calculated 1-MCP or CMP exposures, and calculated Margins of Safety (MOS) for each scenario are shown in Table 2. All calculations and equations used to generate these values were adequately described in MRID 456090-04.

Table 2. Worker Margins of Safety (MOS) for exposure to 1-MCP and CMP

Worker Scenario	Test Material	Total Exposure (70 kg human)	Margin of Safety
Short-term (15-minute)	1-MCP	0.0099 mg/kg	5,656
"Long"-term (4-hour)	1-MCP	0.00792 mg/kg	1,136
Short-term (15-minute)	CMP	0.0000135 mg/kg	>7,000,000
"Long"-term (4-hour)	CMP	0.00216 mg/kg[1]	46,296[1]

[1] Calculated by EPA Reviewer

The rationale used to calculate the MOS of 5,656 for short-term (i.e., 15-minute) worker exposure to 1-MCP is conservative and acceptable, and the MOS is adequate (i.e. >100) to protect workers from potential short-term accidental inhalation exposures.

The rationale for the exposure frequency in the second scenario (i.e., 4-hour exposure to 50 ppb 1-MCP) was unclear. If the 4-hour exposure scenario was intended to be a one-time event, it is unclear why a NOEL based on a subchronic (i.e., 3-month study) was used. For a one-time exposure, however, the calculated MOS of 1,136 would be very conservative. If the 4-hour scenario was intended to apply to repeated exposures, the MOS would be applicable to subchronic exposures. For chronic exposures, an additional uncertainty factor of up to 10 should be applied to the NOEL; if an additional factor were applied, the MOS could be reduced to as low as 113. The MOS of 113 would still provide an adequate margin of safety (i.e., >100) to protect the worker from chronic noncarcinogenic toxic effects resulting from inhalation exposure to 1-MCP.

The approach used to calculate the MOS for CMP, was inappropriate because the LOEL used to calculate MOS was based on carcinogenic effects. Typically, MOS calculations are based non-carcinogenic effects; carcinogenic effects are assumed not to have a threshold (i.e., it is assumed there is some risk associated with any exposure to a carcinogen) Therefore, the calculated MOS does not represent a traditional margin of safety.

EPA Reviewer's Note: Although MOS calculations presented for CMP may be inappropriate for the aforementioned reasons, BPPD concludes that it is highly unlikely that there will be any acute, chronic, and/or subchronic exposure of workers to CMP when the 1-MCP containing products (EthylBloc or SmartFresh) are used according to label directions and workers are wearing the required PPE. When 1-MCP is applied to a storage chamber at the maximum label use rate (1000 ppb or 1 ppm), CMP concentration (if present) will not exceed 0.063% (or 0.63 ppb). Furthermore, the label prohibits workers from being present and/or entering a storage chamber while treatment is in progress, and workers may not re-enter a treated storage chamber until after the chamber has been vented.

III. Consumer Risk to 1-MCP: Consumer risk was assessed by evaluating the potential for chronic and acute dietary exposure to 1-MCP by six population groups (all US adults, all infants <1 yr, nursing infants, non-nursing infants, children 1-6 years, and children 7-12 years). The study author calculated a maximum theoretical residue levels for food commodities using the maximum label use rate, packing density of the commodities in the treatment chamber, and assuming that all of the 1-MCP released in a would be in/on the treated commodity (see MRID 456090-04, p. 12, for an example calculation using apples). Theoretical maximums calculated for apples. pears, avocados, melons, and cucumbers ranged from 4.5 ppb (pears) to 9.0 ppb (apples and cucumbers).

To estimate dietary risk, a "worst-case" residue level of 10 ppb (0.01 mg/kg) for the aforementioned food commodities was assumed (also assuming that 100% of all commodities were treated with 1-MCP and that there was no residue loss with storage, processing, and/or handling). Chronic and acute dietary exposures were calculated for the six population groups (described above) using the Dietary Evaluation Estimation Model (DEEM, software version 7.72, 1994-1996 consumption data). Chronic dietary exposures were evaluated by using the calculated "long-term" NOEL (9 mg/kg/day) obtained from a 3-month inhalation toxicity study (see MRID 456090-01). Acute dietary exposures to 1-MCP were evaluated by comparison to the calculated "short-term" NOEL (56 mg/kg/day) obtained from an inhalation developmental toxicity study (see MRID 456090-08).

The calculated dietary exposures and respective margins of safety for theoretical acute and chronic dietary exposures are presented in Table 3 below.

Population Subgroup	99th Percentile Exposure[1] (Acute)	Acute Margin of Safety	99th Percentile Exposure[1] (Chronic)	Chronic Margin of Safety
Adults	0.000711	78,762	0.000018	500,000
Infants (<1 year)	0.001139	49,166	0.000088	102,273
Nursing infants	0.000944	59,322	0.000043	209,302
Non-nursing infants	0.001138	49,209	0.000101	89,109
Children (1-6 years)	0.001109	50,496	0.000081	111,111
Children (7-12 years)	0.000405	38,272	0.000023	391,304

[1] mg 1-MCP/kg body weight/day

The rationale used to calculate the MOS for acute dietary exposures to 1-MCP is acceptable and the MOS for each population group (range: 49,166 to 138,272) is adequate to protect consumers in each age group against toxicity from acute dietary exposures.

In general, the rationale used to calculate the MOS for the long-term dietary exposure to 1-MCP also is acceptable, and the MOS for each population group (range: 89,109 to 500,000) is adequate to protect consumers in each age group against noncarcinogenic effects from long-term dietary exposures.

The NOELs used to evaluate acute and chronic dietary exposure were both based on inhalation studies. These values were extrapolated to the oral route to evaluate exposure through ingestion. Although data were not available to support the extrapolation, any adjustments that could potentially be made due to route-to-route extrapolation would not be expected to affect the overall conclusions regarding potential risks to consumers.

Potential exposure to CMP was not evaluated for consumers. Based on the information provided in the report, it could not be determined whether consumers also could potentially be exposed to CMP and whether a CMP risk assessment also needs to be performed for consumers.

EPA Reviewer's Note: It is highly unlikely that there will be any consumer exposure to CMP when the product is used according to label directions (applied only to stored food commodities in sealed chambers). Furthermore, the measured ratio of CMP to 1-MCP in a simulated application test was approximately 1:1577 (v/v) inside the chamber. (See MRID 456090-03).

Classification: Acceptable.

SOME OF THE FOLLOWING PAGES CONTAIN CONFIDENTIAL BUSINESS INFORMATION (CBI)

PC CODE: 071297-1 **DP BARCODE: D281146** **MRID NO. 456090-02**

DATA EVALUATION RECORD

1-METHYLCYCLOPROPENE

STUDY TYPE: RESIDUE CHEMISTRY

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
Crystal Station I
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Prepared by

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Primary Reviewer:	Signature:	Nasrin Begum
Nasrin Begum, PhD	Date:	3/12/02
Secondary Reviewer:	Signature:	Renée S. Bouwkamp
Renee Bouwkamp	Date:	3/12/02
Quality Assurance:	Signature:	
Riadh Hossain	Date:	3/13/02

DATA EVALUATION REPORT

EPA Secondary Reviewer: Russell S. Jones Russell S. Jones 5/30/2002

STUDY TYPE:	Residue chemistry (guidelines OPPTS 860.1000 and 860.1500)
MRID NO:	456090-02
CHEMICAL CODE:	224459 Cyclopropene, 1-methyl-(7CI,8CI,9CI)
CASE #	063215
EPA REGISTRATION #	Action 306 (resubmission)
ID #	071297-00001 ETHYLBLOC
SUBMISSION #	S610458
DP BARCODE #	D281146
TEST MATERIAL:	Cyclopropene, 1-methyl- (7CI, 8CI, 9CI) (CA INDEX NAM 0.14%)
PROJECT NO:	Rohm and Haas protocol Number 34P-01-04
SPONSOR(s):	AgroFresh, Inc., A Rohm and Haas Company, 727 Norristown Road, Spring House, PA 19477. Technical Report number AF-01-141
TESTING FACILITY:	AgroFresh, Inc.727 Norristown Road, Spring House, PA 19477
TITLE OF REPORT:	^{14}C-1-Methylcyclopropene (1-MCP) :Apple Residue Study
AUTHOR(S):	D. Verrona, Negro, G., Niemczura, M.
STUDY COMPLETED:	December 12, 2001
CONCLUSION:	This report is consistent with the OPPTS 860.1500 guideline for reporting of data on post harvest fumigation. The study was to determine total radioactive residue (TRR) in apples after treatment with a minimum of 1 ppm (V/V) of ^{14}C-1-MCP, and to demonstrate empirically that residue levels were below 0.01 mg/kg. The study is scientifically sound.
CLASSIFICATION:	ACCEPTABLE
GOOD LABORATORY PRACTICE:	A signed page in the report stating this study was conducted in compliance with FIFRA GLP regulation (40 CFR 160) with

the following exceptions:(1) there was deviation from the protocol and amendment of SOP. (2) the isobutene/air mixture used as GC calibration standards were not GLP characterized (3) the thermometer and the thermocouple probes were not calibrated under protocol, but calibrated against NIST traceable standards. Those anomalies did not adversely affect the scientific merit/validity of the study. Detailed procedural steps including changes made during the study are recorded in the protocol 34 P-01-04, and associated amendments (1 through 10) are included Appendix 1 of the report.

A signed and dated Quality Assurance Statement is provided in the report indicating methods and standard operating procedures and reported results accurately reflect the raw data of the study.

SUMMARY:

This report is consistent with the OPPTS 860.1500 guideline for reporting of data on post harvest fumigation. The study was to determine total radioactive residue (TRR) in apples after treatment with a minimum of 1 ppm (v/v) of ^{14}C-1-MCP, and to demonstrate empirically that residue levels were below 0.01 mg/kg.

The study was conducted on four varieties of commercially important apples: Red Delicious, Fuji, Gala, and Granny Smith. Each variety of apple was treated twice (at two different temperatures). Apples were treated at an exaggerated rate of 1.2 ppm ^{14}C-1-MCP in an airtight chamber for 24 hours. Immediately following treatment the chamber head space was vented to remove airborne ^{14}C-1-MCP and apples were stored in slow air purge. Apple samples were removed periodically (4 hours posttreatment to up to 14 days, depending on variety and temperature) for TRR analysis. A sample set consisted of three apples sampled from the top, middle, and bottom of the apple stack in the chamber, an untreated control and two fortified controls.

The study treatment rate of 1.2 ppm represented a worst case scenario relative to the maximum commercial treatment rate of 1 ppm. At the study treatment rate the theoretical maximum residue concentration was 0.0106 mg/kg. In an additional experiment Red Delicious apples were treated with 1.2 ppm for a week period in order to observe the impact on residue levels of extended exposure beyond the label instructions for treatment time.

Individual treated apple samples were prepared for analysis without sub-sampling or compositing. Analysis for TRR was conducted by liquid scintillation counting. The limit of quantitation (LOQ) for the analytical method was experimentally determined by fortifications to be 0.001 mg/kg and calculated limit of detection (LOD) was 0.0003 mg/kg.

The average fortification recovery for all sample sets was 82.9% with a relative standard deviation of 10.7% (n=39). Average TRR for the eight treatments (four varieties at two temperatures; n=60) was 0.00391 mg/kg (range: 0.00114-0.00911 mg/kg). There were no trends of increasing or decreasing TRR with sampling interval in any apple variety. The TRR in apples treated and stored at ambient temperatures were generally higher than apples treated and stored at 0° C. The highest mean TRR was observed in Gala apples (0.00540 ± 0.00293 mg/kg) treated and stored at ambient temperature. The lowest mean TRR was observed in Red Delicious apples (0.00310 ± 0.00108 mg/kg) treated and stored at 0-3°C. Analytical data were not corrected for method recoveries. Residue levels were calculated as equivalent mg/kg 1-MCP. The found residues represent only 36.9% of the theoretical maximum residue level for the study. The average residue level found in apples treated for a week was 0.00693 mg/kg. It showed even under the relatively extreme conditions of the extended treatment only 65.4% of the available 1-MCP was retained as terminal residue, and the residue level was below 0.01 mg/kg.

I. STUDY DESIGN

Test Material:	^{14}C-1-MCP encapsulated in alpha-cyclodextrin complex
Formulation:	3.16% ^{14}C-1-MCP encapsulated in alpha-cyclodextrin complex
Active Ingredient:	1-methylcyclopropene(1-MCP)
Molecular weight:	54 g/mole
Appearance:	colorless gas
CAS #1-MCP:	3100-04-7
Purity ^{14}C-1-MCP :	94.9%
Specific Activity of ^{14}C-1-MCP:	4.70 mCi/mM; 87.0 mCi/g; 1.93 X 10^{11} dpm/g
CAS# alpha-cyclodextrin:	10016-20-3
Lot #:	1034.00
Specific Activity complex:	3.03 mCi/g
Percent 1-MCP:	3.16%
Appearance:	White powder
Expiration Date:	December, 2001
Reference Material:	Isobutene

METHODS:

A. Treatment Details:

1. Description of Treatment chamber: The chamber was fabricated from aluminum (internal volume 90 liters) designed to be non-reactive (suitable for 0-3 degree C treatment) and

airtight to prevent loss of ^{14}C-1-MCP. Ports were provided for venting after treatment without removing the lid, for the test material introduction, and sampling the chamber air space. The chamber was located in a fume hood. The temperature of the chamber airspace over the apples was checked periodically during treatments using a thermocouple probe/meter.

Apples were placed into the chamber by hand stacking them on top of the perforated shelf at the bottom. The apples were arranged so that the area directly over the circulation fan was left open. The stack was about 30 cm in height at the chamber walls and about 3-4 cm from the lid. The fan pulled in most of the test material from below the shelf after it was introduced and blew it upward onto the bottom of the lid. The turbulence created a fountain effect distributing ^{14}C-1-MCP quickly and evenly keeping uniformity throughout the treatment.

2. Aeration of the apples: Aeration of the chamber was carried out at two points in each treatment, during the vent and storage periods. At the end of each 24 hour treatment period the chamber was vented with air at flow of 40 L/min for a period of 15 to 30 minutes. The chamber was still sealed during the vent and the air was introduced from a side port and expelled from a port on the lid. The above procedure was modified to include sampling of the chamber head space before and during the vent (a description of the modified procedure is included in Appendix 6 of the report). After the laboratory chamber was vented of ^{14}C-1-MCP the air was reduced to 5 L/ min purge. The purge remained on during storage of the apples in the sealed chamber. Apple samples were removed from the chamber periodically depending on each treatment sampling schedule. After each sample was removed from the chamber the chamber was resealed and the purge initiated. There was no aeration of apples after sampling from the chamber. Commercial storage rooms have similar conditions (vent procedures to remove ^{14}C-1-MCP to levels below LOD).

3. Sampling, handling, and storage of apples: After the completion of each laboratory treatment, apple samples were periodically removed from the chamber for analysis based on each treatment's sampling schedule. The sample size was limited to a maximum of three treated apples for each sampling interval. Sample sets consisted of one control, two fortifications and three treated apples. Each sample set consisted of three apples taken from the top, middle, and bottom of the stack. Upon removal from the chamber, each apple was immediately weighed and sealed in sample preparation unit to prevent loss of any volatile residue. Another three apples were removed simultaneously to check core temperatures and these apples were discarded. The lid was removed only those times when apple samples were taken at indicated intervals.

B. Analytical Procedures / Instrumentation

The following describes the summary of the procedures:

Experiment Flow diagram

Remove apple sample set from chamber (top, middle, bottom)

↓

Weigh apples→Seal one apple in each apple preparation unit

↓

Blend apples in saturated ammonium sulfate to homogenize→ fortify untreated apples

↓

Sample 3 aliquots of the head space over the apple homogenate → analyze by LSC

↓

Re-blend homogenate with high speed homogenizer

↓

Filter the homogenate mixture

↓

Sample 3 aliquots of the filtrate → analyze by LSC

↓

Dry the filter cake and grind to homogenize

↓

Sample 5 aliquots of the filter cake → analyze by combustion/LSC

1. Sample preparation: Upon removing apple samples from the chamber each of the three apples were weighed and placed into a separate preparation unit to prevent loss of any volatile residues. Whole apples were prepared without compositing or sub-sampling. The apples were homogenized by blending with 250 ml of saturated ammonium sulfate solution. The salt was present to reduce the solubility of volatile residues in the homogenate and facilitate their release into the head space for easier sampling. In addition, three untreated apples were drawn from the storage freezer and homogenized for a control and two fortifications.

2. Fortifications: Control apple homogenates were spiked with a calculated volume of ^{14}C-1-MCP in air. Two fortifications were prepared for each sample set; one at 0.001 mg/kg and the other at 0.01 mg/kg. The fortification procedure was as follows:
   - A spiking stock mixture was prepared by weighing 20 mg alpha-cyclodextrin and transferring to a Boston round bottom flask with a Miniert valve.
   - Two ml of water was injected into the bottle and the valve was closed. The bottle was swirled to completely dissolve. The concentration of MCP was approximately 1000 ppm.
   - The stock mixture was analyzed by GC/FID according to the method included in the protocol 34P-01-04 (in appendix 1 of the report).
   - Spike volume (for 2 concentrations of MCP) was calculated and spiked by directly injecting the calculated volume into the apple/water mixture, and then was blended for 3 additional minutes.
   - After spiking and blending the fortified controls were allowed to stand for 30 minutes to equilibrate.

3. Sampling a homogenized Apple for Analysis: ^{14}C-1-MCP residue released from a homogenized apple sample in the preparation unit might be found in the head space over the homogenate, dissolved in the liquid component or associated with the solid component of the homogenate. The sampling scheme accounted for all the three possibilities. The head space over the homogenate first sampled directly. The homogenate was then further blended to reduce the particle size of the solids. The homogenate was subsequently filtered to generate a filtrate and a filter cake. The filter cake was dried and ground. Aliquots taken from the head space and filtrate were analyzed by liquid scintillation counting (LSC). Aliquots taken from the filter cake were first combusted to convert ^{14}C residues into CO_2 which was then trapped in an amine/scintillation cocktail solution and analyzed y LSC.

4. Liquid Scintillation Counting (LSC) and Combustion/LSC Analyses: Triplicate aliquots of each head space sub-sample were dissolved in scintillation cocktail for LSC analysis. Five aliquots of each filter cake sub sample were submitted for combustion analysis using a Packard model 307 Sample oxidizer. The oxidizer converted organic ^{14}C into $^{14}CO_2$. The $^{14}CO_2$ was automatically swept from the combustion chamber and trapped into the scintillation cocktail, then submitted for LSC. The LSC program counted all sample aliquots in triplicate for 5 minutes or a maximum total of 200,000 counts. Counting efficiencies were calculated by the external standard channel ratio (ESCR) method with 133 Ba as the external source. Average sample counts per minute (CPM) were determined by dividing the total counting time in minutes into the total number of counts per sample. The equation for calculating DPM is as follows:

Sample DPM = (Avg. Sample CPM-control CPM) X 100 / % counting efficiency.

The software estimated the cpm equivalent to the LOQ and the LOD for each run. If all of the aliquot total dpm values going into the average total dpm were <LOD then the program assigned values to zero. If some of the aliquot total dpm values going into the average total dpm were <LOD the program assigned a value of LOD/2 for averaging. If any of the aliquot total dpm values going into the average were >LOD but <LOQ the program used these values directly for averaging. Average total dpm results <LOQ were used in the calculation of TRR and recoveries. Average total dpm results <LOQ are identified in the Residue Data Tables.

II. RESULTS

A. Main Study-Residue Results: The average residue level determined for the eight treatments (four varieties at two temperatures) in the study was 0.00391 mg/kg. This value was calculated from the residues determined in each apple analyzed (across all four varieties; n=60). This represents only 36.9% of the theoretical maximum (0.0106 mg/kg) residue at the exaggerated rate. Authors indicated that extrapolating from the results of the study, the predicted residue levels in commercially treated apples would be 20% less (0.00323 mg/kg). The apple TRR data are presented in Table 1 for each apple variety and temperature.

Table 1
Detailed Residue Data and Statistics

Treatment	Hours after Vent	Apple Samples / mg/kg residue top	middle	bottom	
#1	4	0.00389	0.00303	0.00436	treatment #1
Red Delicious	26	0.00417	0.00235	0.00415	mg/kg residue
0-3°C	50	0.00146	0.00395	0.00249	average S.D.
	74	0.00370	0.00249	0.00114	0.00310 0.00108
	6 days	0.00351	0.00175	0.00402	
#2	24	0.00555	0.00394	0.00470	treatment #2
Gala	7 days	0.00346	0.00639	0.00453	mg/kg residue
0-3°C	14 days	0.00594	0.00504	0.00447	average S.D.
					0.00489 0.00094
#3	24	0.00259	0.00224	0.00771	treatment #3
Gala	7 days	0.00710	0.00367	0.00911	mg/kg residue
ambient					average S.D.
					0.00540 0.00293

Treatment	Hours after Vent	Apple Samples / mg/kg residue top	middle	bottom	
#4a	zero	0.00598	0.00363	0.00443	treatment #4a
Red Delicious	48	0.00348	0.00532	0.00237	mg/kg residue
ambient					average S.D.
					0.00420 0.00132
#5a	zero	0.00616	0.00279	0.00737	treatment #5a
Granny Smith	48	0.00346	0.00420	0.00529	mg/kg residue
ambient					average S.D.
					0.00488 0.00172
#6a	zero	0.00265	0.00526	0.00339	treatment #6a
Granny Smith	48	0.00331	0.00203	0.00182	mg/kg residue
0-3°C					average S.D.
					0.00308 0.00125
#7	zero	0.00352	0.00362	0.00342	treatment #7
Fuji	48	0.00402	0.00276	0.00474	mg/kg residue
ambient					average S.D.
					0.00368 0.00066
#8	zero	0.00345	0.00319	0.00412	treatment #8
Fuji	48	0.00147	0.00311	0.00142	mg/kg residue
0-3°C					average S.D.
					0.00279 0.00110

B. Main Study-Fortification Results: The average fortification recovery data at the 0.01 mg/kg level was 84.9% with a standard deviation of 7.1%. Average recovery at 0.001 mg/kg level was 80.8% with a standard deviation of 13.5%. Overall average recovery was 82.9% with a relative standard deviation of 10.7%. Residue found in the treated samples were not corrected for recoveries. The limit of quantitation (LOQ) for the procedure was demonstrated by fortification at 0.001 mg/kg. The limit of detection was 0.0003 mg/kg (0.3 X LOQ). Review of residue data table reveals that most of the spiked radioactivity was isolated in the sample filter cakes but some were isolated in the filtrates. Unlike samples significant radioactivity was also found in the head spaces of fortified apples. Recoveries are recorded in the Table 2.

Table 2
Fortification Recovery Tables

Treatment	Hours after Vent	Sample ID	Fort. Level 0.0010 mg/kg % recovery	Sample ID	Fort. Level 0.010 mg/kg % recovery
#1	4	GLP-228-5-2	87.5	GLP-228-5-3	87.3
Red Delicious	26	GLP-228-7-2	95.5	GLP-228-7-3	86.4
0-3°C	50	GLP-228-10-2	73.3	GLP-228-10-3	90.0
	74	GLP-228-13-2	16.6	GLP-228-13-3	86.1
	6 days	GLP-228-15-2	84.8	GLP-228-15-3	103.2
#2	24	GLP-228-22-2	97.5	GLP-228-22-3	86.6
Gala	7 days	GLP-228-25-2	79.7	GLP-228-25-3	76.8
0-3°C	14 days	GLP-228-28-2	82.0	GLP-228-28-3	86.1
#3	24	GLP-228-36-2	94.2	GLP-228-36-3	86.1
Gala@ambient	7 days	GLP-228-40-2	85.2	GLP-228-40-3	83.9
#4a	zero	GLP-240-7-2	81.4	GLP-240-7-3	87.4
Red Delicious	48	GLP-240-13-2	61.7	GLP-240-13-3	75.6
ambient					
#5a	zero	GLP-230-6-2	75.6	GLP-230-6-3	78.3
Granny Smith	48	GLP-230-13-2	92.3	GLP-230-13-3	88.1
ambient					
#6a	zero	GLP-231-6-2	69.5	GLP-231-6-3	83.2
Granny Smith	48	GLP-231-12-2	69.9	GLP-231-12-3	80.0
0-3°C					
#7	zero	GLP-229-7-2	75.2	GLP-229-7-3	77.7
Fuji@ambient	48	GLP-229-12-2	95.2	GLP-229-12-3	80.4
#8	zero	GLP-232-6-2	66.3	GLP-232-6-3	88.0
Fuji@0-3°C	48	GLP-232-11-2	69.7	GLP-232-11-3	87.3

	0.001 mg/kg*	0.01 mg/kg	Overall*
Average	80.9	84.9	82.9
Std. Dev.	10.9	6.1	8.9
RSD	13.5	7.2	10.7
#	19	20	39

3. Extended Treatment Experiment-Residue results: The average residue level determined in the apples undergoing extended treatment was 0.00693 mg/kg. The residue level was 1.8 times higher than the overall average for the main study. Authors' comment was " the higher level is not surprising considering that the apples were exposed to the test material seven times longer than the standard treatment time of 24 hours." They mentioned that not all of the available test material was not absorbed and converted to terminal residue, the 0.00693 mg/kg represents 65.4% of the theoretical maximum. Secondly, the residue level was still below 0.01 mg/kg. Recoveries are recorded in the Table 3.

Table 3
Detail residue Data and Statistics for Extended Treatment (7 days)

		Apple Samples / mg/kg residue				
Treatment	Hours after Vent	top	middle	bottom	Mean	Std. Dev
#9	zero	0.00656	0.00662	0.00679	0.00666	0.00012
Red Delicious	48	0.00667	0.00719	0.00775	0.00720	0.00054.
0-3°C						

Average Residue mg/kg	Min. Residue mg/kg	Max. Residue mg/kg	Number of Samples
0.00693	0.00656	0.00775	6

4. Extended Treatment Experiment - Fortification Recoveries

Fortification recoveries for the extended treatment yielded results similar to those of the main study.

The recoveries are recorded in Table 4.

Table 4
Fortification Recovery Data Treatment #9
Red Delicious Apples @0-3°C Treated 7 days

Hour after Vent	Sample ID	Fort. Level 0.001 mg/kg % Recovery	Sample ID	Fort. Level 0.010 mg/kg % Recovery
zero	GLP-233-10-2	79.1	GLP-233-10-3	83.9
48	GLP-233-13-2	77.5	GLP-233-13-3	82.1

	0.001 mg/kg	0.01 mg/kg	Overall
Average	78.3	83.0	80.7
Std. Dev.	1.1	1.3	2.9
RSD	1.4	1.6	3.6
#	2	2	4

III. DISCUSSION

- The laboratory study was designed to be predictive of residue levels in commercially treated apples. Experimental parameters were similar to commercial treatment conditions (i.e. treatment time, temperature, weight of apple per storage room volume, circulation, and vent).

- The analytical method was the same as the proposed enforcement method and has been adequately validated (LOQ=0.001 ppm).

- A partial list of chromatograms of standards analysis are included (pages 165-184) in the report. Copies of chromatograms of sample analysis were not provided. Therefore, the data could not be reviewed to see if there were any degradation products or other interference present during GC analysis.

- There are discrepancies on dates chamber vented to dates sampled and homogenized. They are as follows:
  1. Page 106 indicates vented 9/19/01, sampled 9/14/01 :48 hour sample
  2. Page 111 indicates vented 9/19/01, sampled 9/26/01 : zero time sample
  3. Page 112 indicates vented 9/19/01, sampled 9/28/01: 48 hour sample.

PC CODE: 071297-1 **DP BARCODE: D281146** **MRID 456090-01**

DATA EVALUATION RECORD

1-METHYLCYCLOPROPENE

STUDY TYPE: 90-DAY INHALATION TOXICITY (RAT)

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
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Date: 3/13/02

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Primary Reviewer: Steven T. Cragg, PhD, DABT
Tetrahedron Contractor
EPA Secondary Reviewer: ~~Roger Gardner, PhD~~ Russell S. Jones
(7511C)

Signed: March 15, 2002
Date 5/30/2002

Data Evaluation Record

STUDY TYPE: 90-Day Inhalation Toxicity (in Rats)
OPPTS 870.3465 & 7800 [§81-3]; OECD Guideline 413 [EEC Directive 92/69/EEC B.2]

DP BARCODE:	D281146	SUBMISSION CODE:	S610458
P.C. CODE:	071297-1	TOX. CHEM. NO.:	224459
MRID No.:	456090-01		

TEST MATERIAL (PURITY): 1-Methylcyclopropene (95.67%-96.20 active ingredient)

SYNONYMS: Cyclopropene, 1-methyl-; 1-MCP

CITATION: Ferguson, J.S., Anderson, D.M., Bernacki, H.J., (2001). 1-Methylcyclopropene: Three-Month Inhalation (Whole-Body) Toxicity Study in Rats. Rohm and Haas Company Toxicology Department. Laboratory Report No. 00R-183. October 19, 2001. MRID 456090-01. Unpublished.

SPONSOR: Rohm and Haas
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EXECUTIVE SUMMARY: In a 90-day inhalation toxicity study (MRID 456090-01), groups of young adult rats (Crl:CD®BR) (10/sex/group) were exposed by inhalation to atmospheres containing 1-methylcyclopropene (1-MCP) for a period of 6 hours/day, 5 days/weeks over the course of 13 consecutive weeks for a total of 67 exposures. Rats were exposed to 1-MCP at target concentrations of 0, 20, 100, or 1000 ppm in whole-body inhalation chambers under dynamic conditions. Actual concentrations were 0, 24 ppm (S.D. ± 5.0 ppm), 107 ppm (S.D. ± 7.4 ppm), or 1031 ppm (S.D. ± 41.1 ppm).

Rats were observed daily for clinical signs of toxicity and for mortality. Once weekly, rats were evaluated using a Detailed Clinical Observations (DCO) assessment. Body weights were recorded on the first day of treatment and once weekly thereafter including the day of sacrifice. Food consumption also was measured weekly. Over exposure days 59-61 (weeks 12 - 13), all rats were subjected to a Functional Observational Battery (FOB) to monitor potential subtle effects on behavior and were given motor activity tests to evaluate potential neurological effects. Ophthalmological examinations were performed prior to exposure and over the last week of exposure. Urinalyses also were conducted over the last week of exposure. After 13 weeks of exposure, rats were sacrificed and necropsied. Blood was collected for hematology and clinical chemistry, selected organs were weighed, and over 40 tissues were processed for microscopic examination.

No treatment-related mortality occurred over the course of the study. Two male rats from the 0 ppm group and one male from the 100 ppm group were found dead over the 13 week exposure period. These deaths were not considered treatment-related because two occurred in the controls and post-mortem examination revealed that the mid-exposure death was associated with a bladder infection after 5 weeks of exposure (no deaths occurred in the high-exposure group). After one week of exposure, routine clinical examination revealed salivation in some rats from the high exposure group immediately after daily exposures, which

disappeared by the next day. Immediate post-exposure salivation recurred in the high-exposure group until the end of the study. In the mid-exposure group, salivation was reported in a single male on one day. Detailed Clinical Observations revealed no treatment-related effects. No changes attributable to treatment were found with the Functional Observational Battery or with motor activity assessments. Body weight, body weight gain, and food consumption were unaffected by treatment with 1-MCP.

Hematology was unaffected by 1-MCP treatment at exposure levels of 100 ppm or less. At 1000 ppm, males and females exhibited decreased red blood cell counts, hemoglobin, and hematocrits. Total white cell counts were elevated in males at 1000 ppm but not females. Mean cell volumes were slightly increased in both sexes at 1000 ppm. Differential white cell counts were not different from controls in males or females at any exposure level. Clinical chemistries were unaffected at exposure levels of 100 ppm or less. At 1000 ppm, 1-MCP caused increased bilirubin and total cholesterol in both males and females and increased triglycerides in females only, indicating an exposure-related affect upon the liver. Urinalyses were unaffected by treatment with 1-MCP at any exposure level. Ophthalmological examination revealed no affect at any exposure level by exposure to 1-MCP.

Organ weights were unaffected by 1-MCP exposures of 100 ppm or less. Relative liver weights were increased in male and female rats exposed to 1000 ppm. Females at this exposure level also exhibited increased absolute liver weights. Both sexes at 1000 ppm had increased absolute and relative spleen weights and females had increased absolute kidney weights. At necropsy, spleens from animals in the high exposure group appeared enlarged in one male and two females. Microscopically, spleens from the high and mid-exposure groups showed increased yellow-brown pigment (hemosiderin) and red pulp congestion in both males and females (and extramedullary hematopoiesis in the high exposure group) . At the 1000 ppm exposure level, livers of both sexes exhibited centrilobular hepatocellular hypertrophy and, in males only, vacuolation of centrilobular hepatocytes. Increased "intracytoplasmic eosinophilic structures consistent with hyaline droplets in the epithelium of the cortical tubules of the kidneys" was evident in males from the 100 and 1000 ppm groups, indicating α-2-microglobulin-type degeneration. "Nuclear enlargement and accumulation of yellow-brown pigment in cortical tubular epithelial cells of the kidneys" occurred in females exposed to 1000 ppm.

The NOAEL for this study is 20 ppm (i.e., actual concentration of 24 ± 5.0 ppm) and the LOAEL is 100 ppm (actual: 107 ± 7.4 ppm), based on histopathological findings in the kidney and spleen. This subchronic toxicity study is classified as acceptable and satisfies the protocol guideline requirements for a subchronic inhalation toxicity study (82-1) in rats (EPA OPPTS 870.3465 "90-Day Inhalation Toxicity" and OECD protocol 413 "Subchronic Inhalation Toxicity: 90-day Study").

COMPLIANCE: Flagging statements (confidentiality, GLPs, and quality assurance) were provided. This study complies with the protocol guidelines provided in EPA OPPTS 870.3465 "90-Day Inhalation Toxicity" and OECD protocol 413 "Subchronic Inhalation Toxicity: 90-day Study." No deficiencies were noted that would compromise the integrity of the study or alter conclusions drawn from results.

I. MATERIALS AND METHODS

A **Materials:**

1. Test Material:	1-Methylcyclopropene
Description:	Colorless gas
Lot/Batch #:	Lot No. RMJ6679A; TD No. 00-0118 (95.80% a.i.) Lot No. RMJ6721B; TD No. 01-0024 (95.67% a.i.)

	Lot No. RMJ6756B; TD No. 01-0054 (96.20% a.i.)
	Lot No. RMJ6766B; TD No. 01-0057 (96.01% a.i.)
Purity:	see above under batch#
CAS #:	3100-04-7
Test Article Analysis:	Gas chromatogram provided in appendices.

2. Vehicle and/or positive control:

No solvent or carrier was used as a vehicle to generate the test atmosphere since the test compound is a gas at room temperature. Air was used as a diluent to achieve the desired concentration.

3. Test animals:

Species:	Rat (males and females)
Strain:	Crl:CD®BR
Age and weight at dosing:	Males: 6 weeks with body weights ranging from 187-232 grams; females: 6 weeks with body weights ranging from 140-179 grams.
Source:	Charles River Laboratories, Kingston, NY.
Acclimation period:	2 weeks.
Diet:	PMI Certified Rodent Diet 5002(M), Purina Mills Inc., Richmond, IN). Food was available *ad libitum* except during 6-hour inhalation exposures and during Functional Observational Battery and motor activity assessments.
Water:	Reverse osmosis-treated municipal water available *ad libitum* except during 6-hour inhalation exposures and during Functional Observational Battery and motor activity assessments.
Housing:	Individually in suspended stainless steel cages (18 x 34 x 18 cm) with wire-mesh fronts and bottoms. Absorbent paper was placed under the cages, which was changed at least 3X/wk.
Identification:	Tail tattoo number.
Environmental Conditions (during non-exposure periods):	
Temperature:	20.6-22.8°C (continuously recorded).
Humidity:	33-58% (continuously recorded).
Air changes:	Not reported.
Photoperiod:	12 hr light/12 hr dark.
Environmental Conditions (during 6-hour exposure periods):	
Exposure Chamber:	240 liter Plexiglass® and stainless steel chamber
Air changes:	At least 19 air changes per hour.
Air flow:	0 ppm chamber: 79.0 L/min ± 0.0 L/min, N=67 exposures) 20 ppm chamber: 51.0 L/min ± 0.0 L/min, N=67 exposures) 100 ppm chamber: 52.0 L/min ± 0.0 L/min, N=67 exposures) 20 ppm chamber: 51.0 L/min ± 0.0 L/min, N=67 exposures)
T_{99}	22 min (based on 51 L/min air flow)
Temperature:	0 ppm chamber: 22.4°C (SD ± 0.6°C, N=67 exposures) 20 ppm chamber: 21.7°C (SD ± 0.5°C, N=67 exposures) 100 ppm chamber: 21.5°C (SD ± 0.6°C, N=67 exposures) 20 ppm chamber: 23.1°C (SD ± 0.6°C, N=67 exposures)
Humidity:	0 ppm chamber: 66.8% (SD ± 8.4%, N=67 exposures)

20 ppm chamber: 72.0% (SD ± 6.7%, N=67 exposures)
100 ppm chamber: 73.4% (SD ± 6.9%, N=67 exposures)
20 ppm chamber: 71.9% (SD ± 6.4%, N=67 exposures)

Photoperiod: 12 hr light/12 hr dark.

B. STUDY DESIGN AND GENERATION/MEASUREMENT OF TEST ATMOSPHERE:

1. In life dates - start: January 30, 2001 end: May 4, 2001

2. Exposure conditions

During exposures, rats were housed in stainless steel wire mesh cages (dimensions not specified) within a 240 liter Plexiglas® and stainless steel inhalation chamber. Assuming rats averaged 500 grams in weight, 1 rat would displace approximately 500 ml total volume. 20 rats per exposure level would displace 10000 ml or 10 liters, which is approximately 4% of the chamber volume (within the guideline of 5%).

3. Animal assignment and treatment

Animals were assigned to the test group noted in Table 1. Rats were exposed to 1-MCP by whole-body exposure for 6 hr/day, 5 d/wk for 13 weeks for a total of 67 exposures (no exposures occurred on weekends or holidays).

Table 1. Study Design

Group	Target Concentration (ppm)	Actual Concentration (ppm ± S.D.)	No./Sex/ Group	No. Exposure Days
1	0	0	10	67
2	20	24 ± 5.0	10	67
3	100	107 ± 7.4	10	67
4	1000	1031 ± 41.1	10	67

4. Generation of the test atmosphere and description of the chamber

Chamber atmospheres were generated by connecting a 1-MCP filled Tedlar Sampling bag through a tube and, using a Gilmont Flowmeter, metering 1-MCP into a 240 liter Plexiglas® and stainless steel chamber containing the test subjects. 1-MCP was drawn into the top of the chamber via negative pressure and mixed with air inside the chamber to achieve the desired concentration. Chamber atmospheres were sampled once hourly. Chamber airflow was monitored continuously. The airflow rate was ~50 liters/minute in the three 1-MCP treatment groups and ~70 liters/min in the air-only control, resulting in >12 air changes per hour, thereby providing at least 19% oxygen within the chambers. The time to t_{99} (equilibration time to reach 99% of target concentration) was less than 10% of total exposure time. Test subjects remained

in the chamber exposed to the target concentration for 6 hours after t_{99} was reached, ensuring 6 hours of exposure to the target concentration.

Test atmosphere concentration

The test atmosphere was sampled once hourly over the 6-hour course of daily exposures. Samples (0.5 ml) were taken from the chamber with a gas-tight syringe and injected directly into a gas chromatograph. A flame ionization detector was used to detect 1-MCP. Results of the analysis are given in Table 1 above. Isobutylene was used as a reference standard by which to measure 1-MCP, rather than 1-MCP itself.

Particle size determination

Particle size determination is not relevant for this gas. Consequently, mass median aerodynamic diameters and geometric standard deviations are not reported in Table 1.

C. METHODS:

1. Clinical Observations

Daily routine observations: Animals were inspected daily for mortality and signs of toxicity. Cage liners were inspected daily for abnormal urine or feces and excess food spillage.

Weekly Detailed Clinical Observations (DCO): Animals were inspected weekly using a checklist to evaluate clinical status in more detail than the routine daily examinations. The parameters monitored are more limited than the Functional Observational Battery (see below) and include:

Home cage	Open arena
Home cage behavior	Number of rears
Home cage involuntary behavior (convulsions or unusual postures or movements)	Presence/absence of fecal boli & condition (e.g., normal, soft, diarrhea)
Reaction to removal from home cage	Number of urine pools
Reaction to handling (transport to arena)	Activity/level of arousal
Lacrimation	Involuntary behavior (convulsions or unusual postures or movements)
Salivation	Gait pattern
Piloerection	Severity of gait abnormality
Respiration rate	Palpebral closure
General appearance	Prominence of the eyes
	Pupillary response (to light stimulus)

2. Body weight

Animals were weighed prior to exposure, on the day of first treatment, and once weekly thereafter including the day of sacrifice. Absolute body weights were recorded for individual animals. Weekly body weight gains were also reported.

3. Food consumption Food consumption was recorded weekly for each rat.

4. Ophthalmic examination

Ophthalmic examinations were conducted on all animals before treatment and during week 13 of exposure. No details were provided about how the procedure was conducted (e.g., Were pupils dilated? What instrument was used to examine the eyes? What parts of the eye were examined).

5. Clinical Pathology

Blood was collected for hematology and clinical chemistries from the abdominal aorta immediately prior to sacrifice from rats anesthetized with sodium phenobarbitol. All subjects were evaluated. Subjects were fasted overnight prior to blood collection. For urinalysis, freshly voided samples were collected from all survivors during week 13 of exposure.

A. Hematology

Except for white cell differential counts and prothrombin times, hematology parameters were measured using a Baker® 9000 Hematology Analyzer (Biochem Immunosystems, Allentown, PA). White cell counts were determined by microscopic examination and prothrombin times were assessed using a IL ACL-1000 (Instrumentation Laboratories, Inc., Milan, Italy). The CHECKED (X) parameters were examined (see table below).

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular hemoglobin (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines.

B. Clinical chemistry

Clinical chemistries were determined with an Hitachi® 704 Random-Access Chemistry System (Roche Diagnostics BoehringerMannheim Corporation, Indianapolis, IN). All subjects were evaluated. Subjects were fasted overnight prior to blood collection. The CHECKED (X) parameters were examined (see table below).

	ELECTROLYTES		OTHER

X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	----------	X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALP)	X	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatinine phosphokinase	X	Albumin/Globulin ratio
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (ALT, aka SGPT)*		
X	Serum aspartate aminotransferase (AST, aka SGOT)*		
X	Gammaglutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines.

C. Urinalysis

Freshly voided urine samples were collected and evaluated during the 13th week of exposure. Specific gravity of the urine was measured with a refractometer, color and clarity was determined visually, sediment was evaluated microscopically, and remaining urinary parameters were determined using Ames Multistix-9® (Miles Inc., Elkhart, IN). All subjects were evaluated. The CHECKED (X) parameters were examined (see table below).

X	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific Gravity	X	Bilirubin
X	pH	X	Blood (occult RBCs and leucocytes)
X	Sediment (microscopic)	X	Nitrate
X	Protein	X	Urobilinogen

6. Behavioral Tests (Functional Observational Battery)

A full functional observational battery (FOB) was conducted to evaluate neurobehavioral performance. These evaluations were performed during the 12th and 13th week of exposure (days 59 - 64). A "primary observer" evaluated the subjects while a "recorder" recorded observations. Observations were made "blind" (i.e., primary observer was unaware of rat's exposure level). The table which follows indicates the parameters evaluated.

Home Cage Observations	Hand Observations	Arena Observations	Manipulations
Posture	Ease removing from cage	Convulsion/tremor/twitch	Approach response
Convulsion/tremor/twitch	Ease of handling	Activity counts	Touch response
Vocalizations	Convulsion/tremor/twitch	Level of arousal	Auditory startle response
Palpebral closure	Salivation/lacrimation	Rearing count	Righting reflex
	Palpebral closure	Grooming	Tail pinch response
	Exophthalmos	Piloerection	Pupil reflex
	Piloerection	Assessment of gait/posture	Grip strength (fore & hindlimb)
	Fur appearance	Record fecal boli/urine	Landing footsplay
	Vocalization on handling		Body temperature

7. Motor Activity

Motor activity was evaluated using passive infrared sensors mounted on a stainless steel cage with a wire mesh front and bottom (41 x 24 x 18 cm). The passive infrared motion sensors (Infrared Motion Activity System, Coulbourn Instruments, Allentown, PA) were calibrated using a calibrator (from Coulbourn Instruments), serving as a mobile, artificial heat source, that mimicked the movement of a rat. Each rat was evaluated for motor activity for 1.5 hours in the apparatus. The number and time spent in movements were counted in 5-minute intervals. A movement lasting less than 1 second was counted as a "small" movement while longer than 1 second was counted as a "large" movement.

8. Sacrifice and Pathology

Three animals died prior to scheduled sacrifice (2 from air-only controls and 1 from the 100 ppm mid-level group). These animals were necropsied and subjected to histopathological examination. All animals sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. All required tissues were examined from the high dose and negative controls only. In addition, livers, kidneys, spleens, and respiratory tracts (nasal cavity, pharynx, larynx, trachea, and lungs) were examined microscopically from the low and mid-exposure groups as well as from the control and high-exposure groups. The XX-marked organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASCULAR/HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary Gland*	XX	Heart*	X	Peripheral Nerve*
X	Esophagus*	X	Bone Marrow*	X	Spinal Cord (3 levels)*
X	Stomach*	X	Lymph Nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic nerve)[T]
X	Jejunum*	XX	Thymus*		_____
X	Ileum*		_____		GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal Gland*
X	Colon*	XX	Kidneys**		Lacrimal Gland [T]
X	Rectum*	X	Urinary Bladder*	X	Mammary Gland [T]
XX	Liver**	XX	Testes**	X	Parathyroids**
N/A	Gall Bladder*	XX	Epididymides	X	Thyroids**
X	Pancreas*	X	Prostate		_____
	_____	X	Seminal Vesicles		OTHER
	RESPIRATORY	XX	Ovaries	X	Bone
X	Trachea*	XX	Uterus*	X	Skeletal Muscle
X	Lung*			X	Skin
X	Nose			X	All gross lesions and masses
X	Pharynx				
X	Larynx				

* Required for subchronic/chronic studies based on Subdivision F Guidelines.
* Organ weight required in subchronic and chronic studies.
[T] Required only when toxicity or target organ.

Three other tissues collected and processed into slides included: cervix, coagulating gland, and vagina. Bone marrow was collected, processed into slides, but not examined. All the tissues listed above with X's, as well as the extra three tissues, were examined microscopically from air-only controls and high exposure Group 4 (1000 ppm) surviving animals (and the three males found dead). Tissues that were examined from low and mid-exposure survivors included: livers, kidneys, spleens, and respiratory tract (nasal cavity, pharynx, larynx, trachea, and lungs).

9. Statistics

Statistical analyses were conducted separately for males and females and using the individual animal as the basic experimental unit. Dichotomous data were evaluated by a Mantel test for trend in proportions and Fisher's Exact test. For continuous data, tests for homogeneity of variance were applied. If non-homogeneous, data were transformed to logarithmic and square-

root values and the test for homogeneity was repeated. If homogeneous, ANOVA was conducted with the F statistic to determine significant difference. If the F statistic was significant, individual groups were compared to controls with Dunnett's test. If non-homogeneous, comparable non-parametric statistical tests were performed. Alphas of $p = 0.05$ or less were considered statistically significant.

II. RESULTS

A. Clinical Observations:

1. Routine Daily Observations - During the second week of exposure, salivation was observed at the end of the exposure period in one male rat at 100 ppm (Group 3) and in a few rats of both sexes at 1000 ppm (Group 4). Salivation was evident immediately upon removal of the rats from the exposure chamber but resolved by the next morning. In the high exposure group, salivation recurred until the end of the study. No other clinical signs of toxicity were reported.

2. Weekly Detailed Clinical Observations (DCOs) - No treatment related effects were detected.

3. Mortality - Two males died from the air-only controls (Group 1). One died in exposure week 5 and the other in week 9. No gross or histopathological lesions were evident at necropsy that may have accounted for either of these two deaths. One male died in the 100 ppm group in week 6 exhibiting "marked hemorrhagic cystitis (of the urinary bladder) with several other secondary inflammatory lesions in other tissues." The authors of the study report did not consider these deaths treatment related because two deaths occurred in the air-only controls, the death in the mid-exposure group appeared to be the result of an infection, and no deaths occurred in the high dose group.

B. Body weight and weight gain

The test material did not affect body weights or weight gain.

C. Food consumption

Food consumption was unaffected by exposure to the test material.

D. Ophthalmic examination

Ophthalmic examinations did not reveal adverse effects upon the eye from treatment.

E. Hematology

At exposure levels of 100 ppm or less, 1-MCP did not affect hematological parameters. At 1000 ppm, red blood cell counts, hemoglobin levels, and hematocrits were reduced compared to controls. RBCs were reduced by 13% in males and 10% in females; hemoglobin levels were reduced by 9% in males and 7% in females; hematocrit levels were decreased by 10% in males and 7% in females. Mean corpuscular volumes were increased slightly but statistically significantly by 4% in males and 3% in females. Males but not females in the 1000 ppm group also had a 40% increase in total white cell counts but white cell differentials (i.e., the proportions of white cells) were unaffected in either males or females.

F. Clinical Chemistries

At exposure levels of 100 ppm or less, 1-MCP did not affect clinical chemistry parameters. At 1000 ppm, statistically significant increases compared to air-only controls were detected for total bilirubin (males 45%, females 46%), total cholesterol (males 36%, females 33%), and triglycerides (females only 50%). Small but statistically significant changes in other parameters were considered spurious and not related to treatment because they did not exhibit a dose-response, were observed in only one sex, and/or were small in magnitude. These included increased total protein (5% in 20 and 1000 ppm males), increased creatinine (26% in 100 ppm males), increased alanine aminotransferase (38% in 20 ppm females), and decreased chloride (2% in 1000 ppm males).

G. Urinalyses

Urinary parameters were unaffected by exposure to the test material at any exposure level.

E. Organ Weights

Concentrations of 100 ppm or less 1-MCP did not affect organ weights. At 1000 ppm, relative liver weights were increased by 12% in males and 23% females and absolute liver weights were increased by 24% in females only. Spleen weights were increased in both sexes exposed to 1000 ppm. Relative spleen weights were increased by 42% in males and 48% in females; absolute spleen weights were increased by 29% in males and 49% in females. Absolute but not relative kidney weights were increased only in females at 1000 ppm by 8%. At slight decrease in absolute but not relative brain weights of 6% found in males was not considered treatment-related because of the small magnitude of the change, lack of a change on a relative-to-body-weight basis, and lack of corresponding histopathology or neuro/behavioral deficits.

F. Gross Pathology

Spleens were enlarged in three rats (one male and two females) from the 1000 ppm exposure group. No other grossly observable pathology was detected in this or lower exposure groups relating to 1-MCP exposure.

G. Histopathology

No microscopic lesions were detected in any animal exposed to 20 ppm 1-MCP. No histopathology occurred in the respiratory tracts (nasal cavity, pharynx/nasopharynx, larynx, trachea, or lung) of any animals at any concentration. Target organs that were affected by 1-MCP exposure at 100 and 1000 ppm included the kidneys and spleen. At the 1000 ppm level only, livers were affected.

Kidneys: In the kidneys of male rats exposed to 100 and 1000 ppm 1-MCP induced lesions in the nephron characterized by "intracytoplasmic eosinophilic structures consistent with hyaline droplets in the epithelium." These changes are consistent with α-2-microglobulin-type degeneration commonly seen in rats exposed to a variety of compounds. In females, kidneys were affected only at the 1000 ppm level and lesions consisted of "nuclear enlargement and accumulation of yellow-brown pigment in cortical tubular epithelial cells and a single occurrence of individual-cell necrosis of tubules with enlarged nuclei."

Spleen: In the spleens of male and female rats exposed to 100 and 1000 ppm 1-MCP, increased yellow-brown pigment (hemosiderin) was detected in primarily the red pulp. This was accompanied by red pulp congestion, and in the 1000 ppm group, increased extramedullary hematopoiesis.

Liver: In the liver, centrilobular hepatocellular hypertrophy was observed in 1000 ppm exposed male and female rats with accompanying vacuolation of centrilobular hepatocytes in some affected males. No similar findings occurred in lower exposure groups.

III. DISCUSSION

A. In this 90-day inhalation toxicity study conducted with rats exposed to target concentrations of 0, 20, 100, or 1000 ppm, 1-MCP caused effects on the kidneys, spleen, and liver. Effects on kidneys may not be biologically relevant to humans since the effects (most pronounced in the males and occurring at 100 and 1000 ppm) were consistent with α-2-microglobulin-type degeneration. This lesion is considered unique to the rat (especially the male), which produces a very high concentration of serum protein globulin thought to be mis-processed by the epithelial cells of the kidney tubule when exposed to high doses of a variety of exogenous chemicals. Histopathological lesions found in the spleen at 100 and 1000 ppm (e.g., extramedullary hematopoiesis, red pulp congestion) were consistent with the gross observation of spleen enlargement and hematology findings of lower RBC counts. Histopathological lesions found in the liver (centrilobular cell hypertrophy) are consistent with P450 metabolic enzyme induction, which also is consistent with increased liver weights. Vacuolation of centrilobular hepatocytes, seen at 1000 ppm in males, is an indication of overt toxicity to this organ. Hepatotoxicity was confirmed at this exposure level by clinical chemistries, which revealed increased bilirubin, cholesterol, and triglycerides. The NOAEL for this study is 20 ppm (actual: 24 ± 5.0 ppm) and the LOAEL is 100 ppm (actual: 107 ± 7.4 ppm) based on effects upon the spleen and kidneys.

B. Deficiencies No deficiencies were found that are expected to affect the outcome or conclusions of this study. Below are some concerns that it might be desirable to clarify.

1. To generate the test atmosphere within the chamber, 1-MCP was not mixed with air to the desired concentration in a plenum prior to introduction of 1-MCP into the inhalation chamber. Rather, pure 1-MCP appears to have been introduced directly into the chamber and then allowed to mix with air to achieve the desired concentration. Repeated 1-MCP measurements were consistent within the chamber but whether samples were taken from the breathing zone was not indicated. Better yet would have been sampling from different locations throughout the chamber showing consistent measurements. Since repeated measurements were taken (once hourly during the exposure period), which showed consistent concentrations, and since a gas such as 1-MCP would be expected to mix quickly and equilibrate with diluent air, introducing undiluted 1-MCP directly into the chamber is not considered serious enough to compromise the integrity of the study. However, it would have been desirable to include a justification for the expected homogeneity of test material within the inhalation chamber. Also, a description would have been helpful describing how animals were housed in the chambers (individually? in groups?) during exposure periods.

2. Isobutylene was used as the standard for 1-MCP. Standard concentrations of 1-MCP were not made and used to generate a calibration curve for the GC. It was assumed that the GC detector would have the same sensitivity for isobutylene as for 1-MCP and that the area under the curve for isobutylene would directly correspond to the concentration of 1-MCP (after adjusting for molecular weight differences). But justification for these assumptions was not provided in the report. Since it is generally recognized that the areas under the curve are consistent for structurally similar chemicals on the same GC column, this assumption is not considered serious enough to compromise the integrity of the study. However, an explanation for why 1-MCP was not used directly to generate a calibration curve (expense?; lack of material?) and why isobutylene was used as a substitute should have been included in the report. Also, an explanation of the relative sensitivities of 1-MCP and isobutylene to the detector and any adjustments for molecular weight differences should also be explained in the report.

3. The report indicates that bone marrow smears were made but not evaluated (see footnote to tissue list, page 30 of report). This is somewhat puzzling given the hematological effects found in blood (where RBCs were decreased and WBCs were increased) and in the spleen (where extramedullary hematopoiesis was occurring). Increased hematopoiesis might have been expected also in the bone marrow.